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Mosquito control applications of fenthion by aerial thermal fog equipment were studied at two sites in Collier County, FL, for sprays that occurred on 20 and 23 June 1984. Acute, lethal effects of fenthion deposited in these estuarine habitats were assessed for caged pink shrimp (Penaeus duorarum), mysids (Mysidopsis bahia), and sheepshead minnows (Cyprinodon variegatus). At Site 1, along a bay with substantial dilution and tidal mixing, fenthion concentrations of 1.5 µg/ℓ and 0.29 µg/ℓ were recorded immediately after both sprays. Concentrations decreased to < 0.020 µg/ℓ 12 h post-spray and no mortality was observed for caged pink shrimp and mysids. Site 2 was along a residential canal system that offered limited dilution and mixing. Measurable concentrations (> 0.038 µg/ℓ) of fenthion persisted at this site for 4 days. Fenthion concentrations in surface waters were toxic to caged pink shrimp and mysids after both sprays; maximum concentrations were 2.6 µg/ℓ and 0.51 µg/ℓ. Caged sheepshead minnows were not affected by the sprays at either site.

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EFFECTS OF AERIAL THERMAL FOG APPLICATIONS OF FENTHION ON CAGED PINK SHRIMP, MYSIDS AND SHEEPSHEAD MINNOWS[1]

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ABSTRACT. Mosquito control applications of fenthion by aerial thermal fog equipment were studied at 2 sites in Collier County, FL, for sprays that occurred on June 20 and 23, 1984. Acute, lethal effects of fenthion deposited in these estuarine habitats were assessed for caged pink shrimp (*Penaeus duorarum*), mysids (*Mysidopsis bahia*) and sheepshead minnows (*Cyprinodon variegatus*). At Site 1, along a bay with substantial dilution and tidal mixing, fenthion concentrations of 1.5 and 0.29 μg/liter were measured in samples taken immediately after both sprays. Concentrations decreased to ≤0.020 μg/liter 12 h postspray and no mortality was observed for caged pink shrimp and mysids. Site 2 was along a residential canal system that offered limited dilution and mixing. Maximum concentrations were 2.6 and 0.51 μg/liter and measurable concentrations (>0.038 μg/liter) of fenthion persisted at this site for 4 days. Fenthion concentrations in surface waters were toxic to caged pink shrimp and mysids after both sprays. No mortality occurred among caged sheepshead minnows at either site.

INTRODUCTION

Fenthion (0,0-dimethyl 0-[3-methyl-4-(methylthio)phenyl]phosphorothioate, Baytex®, Mobay Chemical Corporation) is used as part of an overall program of mosquito control in Lee and Collier counties, Florida. Fenthion is applied from DC-3 aircraft at the rate of 32.5 g/ha by using a 1:20 mixture of Baytex liquid concentrate and diesel fuel. If 100% of this application were deposited and mixed uniformly over an estuarine habitat 0.3 m deep, an aqueous concentration of 11 μg fenthion/liter (ppb) would result. This worst-case prediction exceeds the 24-h LC50 for grass shrimp, *Palaemonetes pugio* (Palaemonidae) (9.5 μg/liter), mysids, *Mysidopsis bahia* (Mysidacea) (0.42 μg/liter) and pink shrimp, *Penaeus duorarum* (Penaeidae) (0.40 μg/liter) (Borthwick et al. 1985).

Since 1980–81, between 38,600 and 53,150 liters of fenthion were used each year in aerial fog and ground ultra low volume (ULV) applications for mosquito control in Florida (Florida Department of Health Rehabilatative Services, Office of Entomology, Jacksonville, FL). Quantitation of environmental concentrations of fenthion is a crucial factor in efforts to relate mosquito control applications of fenthion to effects on non-target aquatic biota. In a study of 4 ground ULV applications of fenthion (11 g/ha) in estuarine saltmarshes (Clark et al. 1985), initial surface concentrations of fenthion ranged from none detected (<0.010 μg/liter) to 0.68 μg/liter (Moore et al. 1985). By 1 h postspray, fenthion concentrations were <15% of the worst-case predictions for ULV applications (3.7 μg fenthion/liter) and no toxic effects were reported for caged grass shrimp and pink shrimp (Borthwick et al. 1985) or benthic communities (Tagatz and Plaia 1985). McKenney et al. (1985) reported lethal or sublethal effects on mysids following two sprays. Wall and Marganian (1971, 1973) applied a granular formulation of fenthion (224 g fenthion/ha) to test plots in a saltmarsh habitat but did not report environmental concentrations of fenthion. Their applications killed fiddler crabs, *Uca* spp., and reduced numbers of amphipods and tanaidaceans, but did not affect other intertidal fauna or caged animals, mostly molluscs and fishes. Studies in freshwater systems have shown that up to 448 g fenthion/ha, applied directly to water, have not caused significant toxic effects on most invertebrates or fishes but did result in significant toxic effects on some aquatic arthropods (Mulla 1961, Patterson and von Windeguth 1964, Linn 1968). Unfortunately, none of the freshwater studies reported measured concentrations of fenthion in water.

Our objective was to determine if mosquito control applications of fenthion are toxic to caged estuarine animals when tested under actual use conditions. Only the toxicity of fenthion is considered in this study because, under the worst-case conditions, the expected concentration of diesel fuel would be 220 μg/liter (or ppb), or approximately one-fifth the 96-h LC50 for mysids (0.88 μliter/liter or ppm; personal communication, Patrick R. Parrish, Gulf Breeze, FL) and larval grass shrimp (1.4 μliter/liter or ppm; Conklin and Rao, 1984). Results of field studies on acute, lethal effects of aerial thermal fog applications of fenthion on caged pink

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shrimp, mysids, and sheepshead minnows, *Cyprinodon variegatus* (Cyprinodontidae), are reported here. These are representative species commonly tested to determine the toxicity of pesticides to estuarine biota (Clark et al. 1986). We compare fenthion concentrations in 2 estuarine sites after 2 mosquito control sprays with worst-case predictions used in hazard assessment of pesticides. The role of estuarine dilution and tidal exchange in reducing fenthion concentrations is discussed; comprehensive, follow-up studies are recommended.

MATERIALS AND METHODS

Study sites and fenthion applications. We studied 2 treated sites within the Collier County, FL, Mosquito Control District and one untreated (reference) site (Fig. 1). Site 1 was within the Isles of Capri development at a homeowner's dock along Johnson Bay, approximately 1 km from Capri Pass and the Gulf of Mexico. At this site, water depth was approximately 1.5 m over a firm sand and clay bottom, covered with a soft marl. This site provided substantial mixing, exchange, and dilution over each tidal cycle because of its proximity to the Bay and Gulf. Temperature, salinity, and dissolved oxygen measurements ranged from 30 to 33°C, 32 to 35‰, and 6.0 to 8.0 mg/liter, respectively, for surface and bottom samples taken during the study. The second site was at a residential dock along a canal within the Marco Island development, approximately 2.5 km by canal from the Marco River. Water sampling and deployments of caged animals were conducted in 1.5–2 m water over a soft silt and muck bottom. The canal, with concrete seawalls on both sides, was approximately 13 m wide with a maximum water depth 4–5 m. Because this site was distant from the mouth of the canal system and the canal system permeated the island development, tidal exchange and dilution with water from outside the canal system was less than that at Site 1. Surface and bottom water quality were similar and ranged from 27 to 30°C and 30 to 33‰. Dissolved oxygen ranged from 5.0 to 8.0 mg/liter (≥100% saturation) except for early morning (0600–0800 h) lows of 3.8 to 4.5 mg/liter readings (56–71% saturation). An untreated (reference) site was located within Rookery Bay National Estuarine Sanctuary. Sampling at this site was conducted from a dock at the Shell Island Field Laboratory on Rookery Bay. The site had a silt and sand bottom, 1.3 m depth, and open tidal exchange with the bay. Temperatures ranged from 29 to 32°C and salinity ranged from 30 to 33‰ in surface and bottom samples at this site. Dissolved oxygen in surface waters ranged from 5.0 to 7.2 mg/liter, however bottom water samples had ≤2.0 mg/liter on several occasions.

Collier County Mosquito Control District personnel applied fenthion at treated sites as part

Fig. 1. Location of study sites in Collier County, FL, during assessment of effects of aerial thermal fog application of fenthion on caged estuarine biota.

of routine control of adult mosquitoes. Although intended for aerial exposures over land, fenthion was deposited in aquatic habitats as a result of drift or by deposition directly below aircraft flying over canals within residential developments. Nominal application rate for aerial thermal fog treatments was 32.5 g fenthion/ha by using a 20:1 mixture of diesel fuel and Baytex Liquid Concentrate (93% active ingredient). Residential areas within our study sites were fogged from DC-3 aircraft between 0630–0730 h on June 20 and 23, 1984. A pilot study at Site 1 on September 13, 1983 provided initial estimates of fenthion concentrations in estuarine waters.

Test animals. Pink shrimp were purchased every other day during the field study from a bait shrimp distributor at Bonita Springs, FL, and transported to the field laboratory at Rookery Bay for acclimation before deployment (Clark et al. 1986). Mortality was <15% during the 12- to 36-h holding period before deployment, except during the night of June 23, when the aeration system failed and approximately 40% of the pink shrimp died. Each morning, 80 shrimp were transported to each study site and placed, 10 per cage, into 4 surface (floating) and 4 bottom cages. Cages were constructed of clear plastic and consisted of 10 compartments with nylon mesh sides for water exchange as described by Borthwick and Stanley (1985). Shrimp length averaged 63 mm and ranged from 44 to 89 mm, rostrum to telson.

Mysids were reared at the Environmental Research Laboratory at Gulf Breeze (ERL/GB), FL, shipped to the field laboratory, and held 2–4 days before deployment (Clark et al. 1986). Mortality during holding was not quantified but was estimated to be <10% based on number shipped to the field site and number available from holding stocks. Mysids, 9–15 days old, were placed in surface cages each morning at test sites. Cages were cylinders of 450 μm nylon mesh with tops and bottoms formed from polypropylene jars (Goodman and Cripe 1987). Three or 4 cages, containing from 7 to 10 mysids each (depending on day and supply of mysids), were deployed daily at each site.

Sheepshead minnows, approximately one month old, from ERL/GB cultures were transported to the field laboratory in the same manner as mysids and held 2 or 5 days. No fish died during holding. Sheepshead minnows were placed in surface cages similar to mysid cages but fitted with 600-μm mesh nylon. Three cages with 10 fish each were set at each site on June 19 and 23.

Field testing of pink shrimp and mysids began the day before the first fenthion spray and continued daily until posttreatment deployments demonstrated no significant mortality (<10%) in 48 h; however, no animals were deployed on June 22. Pretreatment, treatment, and posttreatment survival were compared (ANOVA, SAS 1982) to determine the acute effect of fenthion on caged animals at individual sites. Pretreatment groups were left at each site and exposed with animals deployed on the morning of a spray. Survival of animals at each treated site was compared with that at the untreated site on a daily basis to account for mortality associated with transport, caging effects, or disease. Because expected environmental concentrations of fenthion were orders of magnitude less than laboratory-derived LC50s for sheepshead minnows, fish were deployed only on June 19 and 23, and left for observation for 5 days.

Fenthion analyses. Water samples were collected in 1-liter glass bottles at 12-h intervals, except after a thermal fog treatment when 0.5-, 1-, 2-, 4-, 6-, 9-, 12-, 18- and 24-h posttreatment samples were collected. Triplicate samples were taken at 2-, 4- and 12-h postspray. Surface samples were collected 4 cm below the surface and bottom samples at 10 cm above the bottom at all animal cage sites. Each day, 1 μg of fenthion was added to a 1-liter water sample from the control site and processed with the other water samples. Control and triplicate samples provided estimates of accuracy and precision for measures of environmental concentrations of fenthion.

At the field laboratory, each water sample was drawn through a column of XAD-4® resin at 15 milliliters/min by a peristaltic pump. Columns were held at 4°C and shipped on ice to ERL/GB. There, fenthion was eluted with acetone, extracts were concentrated, and residual water was removed; fenthion was quantitated on a Hewlett-Packard 5710 gas chromatograph with dual N-P detectors according to procedures described by Lores et al. (1985) and Clark et al. (1986). Fenthion detection limit was 0.010 μg/liter; average recovery efficiency was 82 (±10% SD) for field-spiked samples and 98 (±5%) for laboratory-spiked samples.

RESULTS AND DISCUSSION

Fenthion concentrations in water. Fenthion was not detected in water samples taken on June 19, the day before the first spray, at either site. On the morning of June 20, winds were calm or slight at the time of fenthion application. The thermal fog slowly drifted through the treatment areas and a slick of diesel fuel was observed at both treated sites. Development of the slick was enhanced by droplets hitting the water directly beneath the path of the airplanes, apparently an uncombusted diesel fuel and fenthion mixture. The slick persisted for 30–60 min in the area of

caged animals and then dissipated. A slick of diesel fuel was observed again at Site 2 six h postspray. This coincided with a change in tidal flow from an incoming tide that moved water toward the dead end of the canal at the time of spray at Site 2 to an outgoing tide that moved the water in the opposite direction, beginning 5 h posttreatment.

Following spray 1, maximum fenthion concentration at Site 1 was 1.5 μg/liter in the surface water 0.5 h postspray. By 2 h postspray, fenthion decreased to 0.12 μg/liter surface and 0.070 μg/liter bottom, and fenthion was ≤0.020 μg/liter in all samples by 12 h postspray (Fig. 2). At Site 2, a maximum fenthion concentration of 2.6 μg/liter was recorded at 0.5 h postspray in the surface sample; the bottom sample contained 0.12 μg/liter. Although fenthion concentrations decreased in surface waters over the next 2 h, a change in tidal flow brought increasing concentrations of fenthion to the area of caged animals from 2 to 6 h postspray; concentrations peaked at 1.4 μg/liter at 6 h postspray. By 8 h postspray, surface water concentration of fenthion was 0.51 μg/liter and concentrations diminished from 0.46 to 0.053 μg/liter over the subsequent 60 h. In bottom waters, fenthion concentrations slowly increased from 0.082 μg/liter at 1 h postspray to 0.15 μg/liter at 24 h postspray. Fenthion concentrations at the bottom remained constant at 0.15 μg/liter over the next 24 h, then decreased to 0.036 μg/liter over the 24 h before spray 2.

On the morning of the second spray (June 23), 7–13 km/h winds rapidly moved the fenthion thermal fog through the treated areas. No diesel slick was observed at Site 1, but a slick persisted at Site 2 for 20 to 30 min. Maximum fenthion concentrations of 0.29 μg/liter surface and 0.034 μg/liter bottom were recorded at 6 h postspray at Site 1 (Fig. 2). By 24 h postspray, no fenthion was detected in water samples from Site 1. At Site 2, fenthion concentrations peaked in the surface water 0.5 h postspray at 0.51 μg/liter, then slowly decreased over the next 4 days. In the bottom water, fenthion increased from 0.036 μg/liter to 0.29 μg/liter during the first 6 h postspray, then slowly decreased to 0.038 μg/liter over the subsequent 4 days.

Dilution by surrounding water and tidal exchange at Site 1 kept fenthion exposures to <12 h duration. This was also observed during our

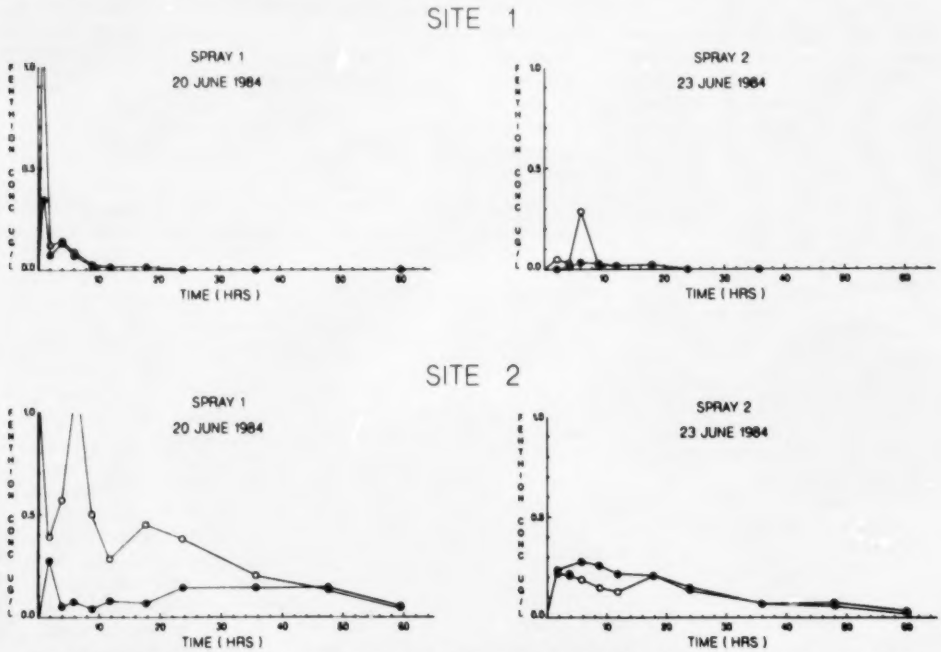

Fig. 2. Concentrations of fenthion measured in surface (open circles) and bottom (closed circles) water samples 2 h to 60 h postspray at 2 study sites after 2 aerial thermal fog applications of fenthion. Upper figures are for Site 1, lower figures for Site 2. Detection limit for fenthion was 0.010 μg/liter. Initial surface concentrations (0.5 h postspray) were 1.5 μg/liter at Site 1 and 2.6 μg/liter at Site 2 after spray 1 and <0.02 μg/liter at Site 1 and 0.51 μg/liter at Site 2 following spray 2.

pilot study when initial fenthion concentrations of 0.43 μg/liter were reduced to 0.040 μg/liter by 12 h postspray. Fenthion applications were intended for areas over land adjacent to open waters and fenthion that entered the aquatic habitat was quickly diluted, reducing animal exposures at Site 1. Limited water exchange and mixing within the canal at Site 2 caused fenthion concentrations in surface and bottom waters to converge over 48 h postspray. Also, animal exposure to fenthion in the canal system for 4 days after a spray was the result of fenthion thermal fog sprayed over the entire canal system on Marco Island and the limited exchange with uncontaminated water within the canals.

Effects on caged animals. Animal survival at the reference site indicated no severe caging, acclimation, or disease problems among batches of test organisms. Mysid survival was ≥81% over 72 h for all batches, range 81% to 93%. Survival of pink shrimp in surface cages ranged from 95% to 100%. A survival rate of 76% was recorded for 4 batches of pink shrimp held for 72 h in cages along the bottom at the reference site. Survival for the other 2 batches was 95% (deployed June 24) and 58% (deployed June 25). The relatively lower survival rates for pink shrimp in bottom cages was attributed to low dissolved oxygen, ≤2 mg/liter, in deeper areas near mangrove roots. These problems highlight the difficulty of finding a reference site with water quality conditions identical to that of treated areas. Also, they reinforce our decision to emphasize animal survival rates at pretreatment, treatment, and posttreatment intervals within a test area.

No mortality attributable to fenthion occurred among caged pink shrimp, mysids, or sheepshead minnows at Site 1 (Table 1). Survival through 72 h was ≥94% for all groups of test animals at this site. Although initial fenthion concentrations after the two aerial thermal fog applications exceeded 24-h LC50s for pink shrimp and mysids, the concentrations did not persist long enough to cause acute mortality (Clark et al. 1986).

At Site 2, animal exposures to fenthion after aerial sprays were prolonged because of limited mixing and tidal exchange. By 24 h after spray 1, no surface pink shrimp were alive, and only 22% and 56% of exposed mysids had survived (Table 2). At 48 h postspray, mysid survival was reduced to 6% and 13%, respectively. All animals held at Site 2 for 24 h before spray 1 survived, and survival for 24 h beginning the day after spray 1 was 80% for surface pink shrimp and 94% for mysids.

No surface sheepshead minnows died at Site 2 after spray 1. No fish mortality was expected because fenthion concentrations were <0.001 of the LC50 for sheepshead minnows (Clark et al. 1986). Although fenthion was detected in bottom waters at Site 2, concentrations did not exceed pink shrimp LC50s and no significant mortality occurred among caged pink shrimp on the bottom.

Survival of pink shrimp at Site 2 24 h after the second spray was 18% in surface cages and

Table 1. Daily survival percentages for caged mysids and pink shrimp deployed at Site 1 to assess effects of aerial thermal fog applications of fenthion in Collier County, FL, in 1984. Spray 1 occurred on June 20, spray 2 on June 23.

Test animal [position]	Number animals per batch	Date of observation (June)								
		Spray 1				Spray 2				
		19	20	21	22	23	24	25	26	27
Mysids										
(*Mysidopsis bahia*)	32	*	97%	94%	94%	94%				
[surface]	28		*	95%	95%	95%				
	32			*	100%	100%	96%	83%		
	30					*	100%	93%	93%	
	30						*	100%	100%	97%
Pink Shrimp										
(*Penaeus duorarum*)	40	*	100%	100%	100%	100%				
[surface]	40		*	98%	98%	98%				
	40			*	100%	100%	100%	100%		
	40					*	100%	100%	100%	
	40						*	100%	100%	
Pink Shrimp	40	*	100%	100%	100%	100%				
[bottom]	40		*	100%	100%	100%				
	40			*	100%	100%	100%	100%		
	40					*	100%	100%	100%	
	40						*	100%	100%	

* Denotes day of deployment.

Table 2. Daily survival percentages for caged mysids and pink shrimp deployed at Site 2 to assess effects of aerial thermal fog applications of fenthion in Collier County, FL, in 1984. Spray 1 occurred on June 20, spray 2 on June 23.

Test animal [position]	Number animals per batch	Date of Observation (June)								
			Spray 1			Spray 2				
		19	20	21	22	23	24	25	26	27
Mysids										
(*Mysidopsis bahia*)	32	*	100%	22%	6%					
[surface]	32		*	56%	13%					
	32			*	94%	84%	16%	13%		
	30					*	83%	60%	47%	
	30						*	97%	93%	90%
	30							*	100%	97%
Pink Shrimp										
(*Penaeus duorarum*)	40	*	100%	0%						
[surface]	40		*	0%						
	40			*	80%	48%	0%			
	40					*	18%	0%		
	40						*	74%	41%	
	40							*	100%	98%
Pink Shrimp	40	*	100%	93%	90%					
[bottom]	40		*	95%	95%	90%				
	40			*	100%	100%	41%			
	40					*	82%	38%	15%	
	40						*	80%	50%	
	40							*	98%	93%

* Denotes day of deployment.

82% in bottom cages and 0% and 38%, respectively, at 48 h postspray. Beginning 2 days after spray 2, survival of caged pink shrimp and mysids was ≥93% for 48-h exposures. Most of the mysids (90%) set out the day after spray 2 survived the subsequent 72 h. Pink shrimp set out the day after spray 2 had been stressed by lack of aeration during the previous evening's holding, and their survival at Site 2 was less than expected, 41% surface and 50% bottom over 48 h. However, survival for this batch of pink shrimp was 100% at Site 1 and 95% at the reference site over the same time interval. All sheepshead minnows survived at Site 2 following the second spray.

Because mortality was less than 20% in pretreatment and posttreatment animals held in the absence of fenthion, mortality of caged pink shrimp and mysids at Site 2 is explained by prolonged exposure to lethal concentrations of fenthion (Clark et al. 1986). None of the fenthion concentrations approached lethal exposures for sheepshead minnows. Neither the extremes nor rate of change of temperature, salinity or dissolved oxygen concentrations seriously affected the test animals as evidenced by the high survival rate (≥80%) when fenthion was nondetectable or present at low concentrations. Unexpected mortality among pink shrimp deployed on June 24 was attributed to a possible interaction between stress from previous exposure to low dissolved oxygen and fenthion exposure (Clark et al. 1986).

Implications for effects on estuarine biota. Because the studies were limited to effects on caged animals, our results cannot be applied directly to effects on resident estuarine communities. Extrapolation of our data may underestimate potential impact on plankton communities because we did not quantify fenthion in contaminated water masses. Rather, we sampled at a fixed site as water moved past. We limited our assessments to acute effects of two applications of a mosquito control program that utilized aerial thermal fog applications throughout the mosquito breeding season. On the other hand, by denying our caged test animals an opportunity to avoid contaminated water and by holding them in the water column rather than allowing them to move to bottom water, we may have overestimated the effects of aerial thermal fog applications of fenthion on crustaceans.

Concerns over the potential for aerial thermal fog applications of fenthion to cause adverse effects on estuarine biota appear to be justified as each spray resulted in initial fenthion concentrations that exceeded acute LC50s for mysids and pink shrimp. However, a comprehensive study would be necessary to assess the degree to which that potential is realized for nontarget estuarine communities. Such a study must evaluate the role of various factors that might en-

hance or mitigate animal exposures to fenthion, e.g., mixing and dilution characteristics of local habitats, weather conditions, potential for cumulative effects of repeated applications over the summer, animal movements and behavior. We believe that a comprehensive follow-up study is warranted, but we recognize that any attempt to address these confounding factors will require a study of much larger scale.

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